# BACTERIOLOGIC STUDIES OF GASTRIC FRACTIONS OBTAINED BY THE REHFUSS METHOD

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The fractional method of gastric analysis makes possible a study of the stomach contents at the different stages in the process of digestion and in the resting or "interdigestive" phase as well. Until recently this method has been employed almost exclusively for obtaining chemical data which would be of assistance to the clinician. However, this method can likewise be used for the determination of the bacterial content of the stomach. So far as could be ascertained, no quantitative studies concerning numbers of bacteria present in various gastric fractions have hitherto been reported. In fact little is known with regard to the types of bacteria to be found in these fractions beyond the work of Cotton [1] who claims that, "The stomach and duodenum are very frequently the seat of secondary foci. . . . The bacteria invade the stomach wall and appear to interfere with the secretion of hydrochloric acid, so necessary to digestion. Cultures of the stomach contents will reveal the presence of various types of streptococci and frequently of various types of colon bacilli. The chemical examination of the stomach contents will show either a very low secretion of hydrochloric acid, or in many cases, its entire absence during the test meal." On the administration of autogenous vaccines the acidity of the stomach is said to be increased and the bacteria to disappear.

The first criticism which can be advanced against such a position is that these conclusions are based on single determinations by the Rehfuss method of fractional analysis. I have shown [2] that repeated analyses carried out on the same person within a short period of time yield different acidity curves. In other words, the same subject may show a low, high and intermediate acidity on three separate analyses carried out within a single week. Obviously, therefore, it is not valid to base any conclusions on a single determination.

The Rehfuss method of fractional gastric analysis was carried out in the usual way on a number of healthy and psychotic persons. The

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[1] New York Med. Jour., 1920, 111, pp. 672, 721 and 770. The Defective Delinquent and Insane, p. 201.

[2] Nicholas Kopeloff: Jour. Am. Med. Assn., 122, 78, p. 404.

modifications introduced were as follows: Sterilization of the Rehfuss tubes and syringes; rinsing of the mouth with chlorazene solution (1 teaspoonful of powdered aromatic chlorazene to a cup of sterile water) followed by several thorough rinsings with sterile distilled water. The conditions for bacteriologic study, consequently, were somewhat more aseptic than those generally employed. Broth tubes were inoculated with the gastric fractions during analysis and incubated for 24 hours at 37½ C. Gram stains were made of all fractions. From these broth cultures streaks were made on lactose meat infusion agar having brom cresol purple as an indicator, and pure colonies were fished therefrom. Holman[3] was followed for the classification of streptococci, and Chester[4] for all other organisms. In all, a qualitative study was made of about 1,000 gastric fractions.

TABLE 1

BACTERIAL SPECIES FOUND BY THE FRACTIONAL METHOD OF GASTRIC ANALYSIS

| | |
|---|---|
| Staphylococcus albus | Bacterium bossonis |
| Staphylococcus aureus | Bacterium acidiformans |
| Staphylococcus citreus | Bacterium ambiguum |
| Streptococcus viridans | Bacterium aerogenes |
| Streptococcus pyogenes | Bacterium mycoides |
| Streptococcus fecalis | Bacterium I |
| Streptococcus equinus | Bacterium II |
| Streptococcus salivarius | Bacillus vulgatus |
| Streptococcus mitis | Bacillus dendriticus |
| Streptococcus ignavus | Bacillus vulgaris |
| Streptococcus subacidus | Bacillus cloacae |
| Enterococcus | Bacillus coli |
| Bacterium lacticum | Bacillus I |
| Bacterium acidi-lacti | Bacillus II |
| Bacterium viscosum | Yeasts, nonchromogenic |
| Bacterium desidiosum | Yeasts, chromogenic |

However important such a qualitative bacteriologic investigation may be, it soon becomes apparent that quantitative results are of equal, if not greater, significance. Invariably, infection connotes numbers of bacteria. Therefore, in addition to classifying the bacteria found in the various gastric fractions, an attempt was made to determine the actual number of bacteria present at each stage of the analysis. The procedure was as follows: One c c of each gastric fraction on withdrawal was at once plated out in duplicate (at the bedside) on lactose meat infusion agar. The plates were incubated in the usual way and counted in 24 hours, and again in 3 days.

Another factor of considerable importance in the proper interpretation of analyses by means of the Rehfuss method is the saliva. Varying

[3] Jour. Med. Res., 1916, 29, p. 377.

[4] A Manual of Determinative Bacteriology, 1902, p. 401.

quantities of saliva are swallowed by different persons, and it is of importance to determine the bacterial flora of the saliva in question, as well as to note the amount swallowed. Careful record, therefore, has been kept of the numbers of bacteria occurring in composite samples of saliva obtained during the analysis, as well as of the types found, and the chemical reaction.

In table 1 will be found a list of the species of bacteria isolated from the various gastric fractions. The streptococci and staphylococci are well represented, as are the lactobacilli. Many of the listed names represent approximations due to atypical characters of the organisms, rather than constituting exact species. Thus it has been necessary to call several atypical strains of nonhemolytic streptococci: Streptococcus viridans. It will be noted, however, that the great majority of these organisms are generally regarded as nonpathogenic. Many of them are, to be sure, facultatively pathogenic and merely await a favorable opportunity to become true pathogens. The species found are in close agreement with some unpublished data, very kindly placed at our disposal by Dr. L. W. Famulener.

The occurrence of these organisms in the various gastric fractions is shown in table 2, which gives a summary of data to be given in detail elsewhere.[5] The high acidity average is an average of the 3 highest total acidity values obtained during the analysis of that date; the average number of bacteria is the average found concomitantly with the acidities just mentioned; the low acidity average is the average of the 3 lowest total acidity values obtained in the same analysis; the average number of bacteria found concomitantly with those acidities; and the last column gives the names of these types of bacteria. The table itself is divided into 2 parts, the first half being the results obtained with healthy normal persons and the second half being devoted to psychotic patients. These results have been greatly condensed and these subjects may be considered representative of the larger group studied.

Certain rather broad features can be clearly distinguished. In the first place, if one glances down the column of types of bacteria found for the three highest total acidities and compares them with the types found for the lowest total acidities during the same analysis, a marked similarity will be discerned between the parallel columns at almost every point. This holds true for psychotic as well as for normal persons. For example, in the very first subject, C.Te., on August 31, when the

[5] Nicholas Kopeloff: State Hosp. Quart. (N. Y.), May, 1922.

TABLE 2

BACTERIA IN GASTRIC FRACTIONS OF HIGH AND LOW ACIDITY

| Name | Date | Average Three High Acid | Average No. Bacteria | Types | Average Three Low Acid | Average No. Bacteria | Types |
|---|---|---|---|---|---|---|---|
| | | | | Normal | | | |
| C. Te. | 8/31 | 64 | 47 | Yeasts | 24 | 46 | Yeasts, S. fecalis |
| | 9/ 2 | 68 | 25 | S. viridans, Bact. ambiguum | 30 | 14 | S. viridans, Bact. ambiguum |
| | 9/ 6 | 81 | 3 | Yeasts, Bact. ambiguum | 45 | 2 | Yeasts |
| S. Mc y. | 8/31 | 43 | 943 | Yeasts, Bact. lacticum, S. viridans, Bact. ambiguum | 9 | 30,000 | B. coli, S. viridans |
| | 9/ 2 | 72 | 1,166 | Bact. ambiguum, S. viridans, yeasts | 23 | 720 | Bact. ambiguum, S. viridans, yeasts |
| | 9/ 6 | 84 | 428 | Bact. ambiguum, S. viridans, yeasts | 30 | 405 | Bact. ambiguum, S. viridans, yeasts |
| M. Mc a. | 8/31 | 52 | 0 | S. viridans, yeasts | 25 | 27,000 | S. viridans, yeasts |
| | 9/ 2 | 64 | 0 | Nil | 40 | 1 | Bact. mycoides |
| | 9/ 6 | 64 | 0 | Enterococcus | 46 | 1 | Enterococcus |
| | | | | Psychotic | | | |
| E. Zn. | 7/ 7 | 28 | 22 | Bact. lacticum, Bact. mycoides, B. vulgaris | 9 | 15,434 | Bact. mycoides |
| | 7/21 | 23 | 8 | B. cloacae, yeasts | 7 | 78,000 | B. cloacae |
| M. Sl. | 3/14 | 44 | 68 | S. viridans, S. albus, yeasts, Bact. lacticum | 18 | 5,000 | S. albus, S. viridans, yeasts |
| | 3/16 | 39 | 2 | Bact. aquatilis, S. albus, yeasts, Bact. lacticum | 8 | 4,500 | S. albus, S. fecalis, Bact. acidi-lacti, yeasts |
| | 6/28 | 38 | 64 | B. vulgatus, yeasts, S. mitis | 8 | 21,518 | B. vulgatus, S. mitis |
| | 11/16 | 43 | 18 | S. mitis | 13 | 5,533 | S. mitis |
| I. Sn. | 7/29 | 48 | .... | S. aureus, S. fecalis, yeasts | 16 | ...... | S. aureus, S. fecalis, yeasts |
| | 8/14 | 40 | 285 | S. aureus | 21 | 436 | S. aureus |
| M. Hr. | 6/30 | 56 | 39 | Bact. lacticum, yeasts | 31 | 16 | Bact. lacticum, yeasts |
| | 7/ 7 | 63 | 31 | Bact. lacticum, yeasts | 24 | 49 | Bact. lacticum, yeasts |
| | 7/14 | 51 | 12 | S. aureus, yeasts | 22 | 6,335 | S. aureus, yeasts |
| E. Kg. | 4/19 | 57 | 11 | S. albus, Bac. I, yeasts | 43 | 3 | S. albus, Bac. I, yeasts, S. salivarius |
| | 6/30 | 67 | 1,517 | Bact. lacticum | 34 | 623 | Bact. lacticum |
| E. Mc y. | 7/ 7 | 24 | 618 | Bact. lacticum | 9 | 15,000 | Bact. mycoides, Bact. lacticum |
| | 7/14 | 22 | 32 | S. aureus, Bac. I | 11 | 4,133 | S. aureus, yeasts |
| | 11/ 4 | 22 | 203 | Bact. | 10 | 4,650 | Bact. |
| M. Dn. | 7/ 7 | 25 | 17 | S. aureus, Bact. lacticum, yeasts | 16 | 8,513 | Bact. lacticum, yeasts |
| | 7/21 | 67 | 308 | S. aureus, Bact. lacticum, yeasts | 23 | 591 | Bact. lacticum, S. aureus |
| | 7/26 | 55 | 693 | Yeasts | 15 | 320 | Yeasts |
| I. Be. | 4/19 | 78 | 2,966 | Bact. lacticum, yeasts | 21 | 20,430 | Bact. lacticum, yeasts, Bact. I |
| | 4/26 | 72 | 118 | Bac. I, yeasts | 41 | 87 | S. albus, B. vulgatus, Bact. I |
| | 6/28 | 62 | 113 | S. ignavus, Bact. lacticum, yeasts | 39 | 885 | S. albus, Bact. lacticum, yeasts |
| B. Cy. | 3/16 | 50 | 19 | Bacterium acidi-lacti, yeasts, Bact. ambiguum, S. ignavus | 26 | 43 | Bact. I, yeasts, S. ignavus |
| | 4/19 | 55 | 2 | Yeasts | 19 | 2,420 | S. albus, yeasts |
| | 4/26 | 54 | 58 | Bac. I, yeasts | 10 | 3,176 | S. albus, B. vulgatus |
| M. By. | 7/14 | 64 | 450 | S. aureus, yeasts | 24 | 53 | S. aureus, yeasts |
| | 8/14 | 95 | 61 | Yeasts | 21 | 1,064 | S. aureus, yeasts, S. mitis |
| T. Cd. | 6/30 | 51 | 3 | Bact. lacticum, S. mitis | 27 | 4 | Bact. lacticum, S. mitis |
| | 7/21 | 46 | 1 | Bact. lacticum, yeasts | 19 | 6,040 | Bact. lacticum, S. aureus, yeasts |
| | 7/26 | 63 | 3 | Bact. mycoides, S. viridans, yeasts | 31 | 175 | Bact. mycoides, S. viridans |

average high total acidity was 64, and 47 bacteria per c c were found, yeasts were present. The average low total acidity was 24, and the number of bacteria 46. Again yeasts were found, together with S. fecalis. On September 2, with an acidity of 68, 25 bacteria were found, the types being S. viridans and Bact. ambiguum. With the low acidity of 30 there were only 14 bacteria comprising identically the same types as those just mentioned. Such close parallelism occurs with great frequency throughout both series.

This observation is of considerable significance, for it shows rather convincingly, that acidity is not of prime importance with regard to bacterial species found. In other words, instead of finding a great many more delicate organisms at the lower acidities, we find virtually the same flora as occurs at higher acidities. This is further emphasized by the fact that the flora remains the same, irrespective of the numbers of bacteria present at the differing acidities. It will be noted that there is great inconsistency in the bacterial numbers at different acidities. In some instances, notably C.Te., one actually finds fewer bacteria with lower acidity, which is contrary to expectation. Again this would indicate that acidity is not the limiting factor as far as the bacterial content of the stomach is concerned. This will warrant further discussion in connection with other quantitative results still to be considered. It is interesting to observe that the more common organisms appear in the same person at different times, but this occurrence is scarcely any more regular than the occurrence of the same species in different persons on the same day—indicating that external factors are fully as important as the internal factors. However, the fact that the more uncommon organisms are not found with any degree of regularity, either in the same subject or on the same day, makes it inadvisable to draw any inferences, but rather to consider these phenomena as being instances of the law of probabilities.

Concerning the occurrence of streptococci, it will readily be seen that in general gastric acidity does not appear to be of much concern to them. Thus in the subject showing the lowest gastric acidity, namely, E.Zn. (the first of the psychotic patients)—in whom the highest average acidity is below 30—no streptococci were found. On the other hand, in the first two normal subjects, C.Te., and M.Mca., Streptococci viridans occurs in conjunction with high gastric acidity. Thus there is little to be said for the close association of streptococci with low gastric acidity, and still less for its importance as an etiologic factor in the psychoses, considering its cosmopolitan appearance in

normal persons. The same may be said for B. coli, which occurred, not in the subject with the lowest acidity, but in the one showing the highest acidity. Incidentally, it may be mentioned that this subject was a healthy young nurse rather notorious for her good digestion. Finally, it must be stated that almost invariably the same organisms found in the stomach were isolated from the saliva and the test meal. The former supplied the members of the streptococcus and staphylococcus groups, and the toast accounted for the remaining yeasts and bacteria.

All the considerations, therefore, point to the conclusion that bacterial species found by the Rehfuss method occur independently of the acidity present, and that there is no correlation in psychotic patients between low acidity and a development of streptococci or B. coli. Consequently, under such conditions it cannot validly be asserted that the stomach (without lesions) is a focus of infection.

Turning now to a consideration of bacterial numbers, it has been stated that in only one half of the number of instances has there been found a correlation between high acidity and low bacterial numbers or vice versa. This, together with the observations on the bacterial species found in the saliva and test meal, indicated that the latter relatively external factors were of greater importance in determining the bacterial content of the stomach than was the gastric acidity. In this connection it may be mentioned that the only subject who showed an absence of bacteria in the stomach on repeated analysis was a patient with profound depression. Her mouth was exceedingly dry, and therefore the absence of saliva was regarded as the limiting factor.

A method was finally devised for conducting a fractional gastric analysis in such a manner as to isolate the saliva as a factor for special study. A dental suction tube attached to a running water pump was inserted in the patient's mouth throughout the analysis. This reduced the swallowing of saliva to a minimum. An attempt was made to have all conditions as aseptic as the circumstances would permit. In this way, counts made on the bacteria would be a true approximation of the numbers and kinds actually present in the stomach and could be satisfactorily compared with previous results obtained when saliva was present in greater amount.

In table 3, are presented the bacteriologic and chemical results obtained with a psychotic patient (diagnosed manic-depressive: manic). The first two main headings "Saliva Not Removed" are for the fractional gastric analyses carried out at two different times with the technic employed in previous work. Under the final heading "Saliva Removed"

appear the data obtained with the modified technic described. In the first column of figures giving bacteria per c c, when saliva was not removed, we see that the fasting contents contained 15,500 bacteria per c c. During the process of digestion the numbers are considerably lower until 2¼ hours have elapsed, when there is a tremendous increase. This occurs despite the fact that the acidity in the stomach is generally sufficient to decimate a large proportion of the original number of bacteria ingested. Contrast the first two columns of bacterial numbers when saliva was not removed with the column of bacterial figures when saliva was removed. In the latter column, the first number is 2, and the last, which is the highest, is 32. The results speak for themselves. Such a striking reduction makes the conclusion irresistible: namely, that bacterial numbers in the stomach depend almost entirely on the saliva

TABLE 3
INFLUENCE OF SALIVA

| M. Sl. | Saliva Not Removed | | | Saliva Not Removed | | | Saliva Removed | | |
|---|---|---|---|---|---|---|---|---|---|
| | Bacteria per 1 C c | T A | pH | Bacteria per 1 C c | T A | pH | Bacteria per 1 C c | T A | pH |
| F. C. | 15,500 | 5 | 2.8 | ..... | .. | ... | 2 | 12 | 2.8 |
| ¼ hour | 380 | 11 | 2.7 | 310 | 35 | 2.9 | 5 | 23 | 2.5 |
| ½ hour | 78 | 41 | 2.2 | 5,100 | 37 | 3.0 | 8 | 52 | 1.7 |
| ¾ hour | 5 | 46 | 1.8 | 925 | 41 | 2.8 | 2 | 70 | 1.4 |
| 1 hour | 60 | 25 | 2.5 | 2,800 | 28 | 3.0 | 0 | 71 | 1.3 |
| 1¼ hours | 800 | 18 | 2.7 | 110 | 36 | 2.2 | 1 | 42 | 1.5 |
| 1½ hours | 215 | 25 | 2.3 | 95 | 43 | 1.9 | 1 | 29 | 1.7 |
| 1¾ hours | 55 | 6 | 2.7 | 12 | 45 | 1.8 | 32 | 12 | 2.0 |
| 2 hours | 110 | 28 | 2.7 | 5,400 | 10 | 3.7 | * | 2 | 3.0 |
| 2¼ hours | 48.000 | 9 | 2.7 | 3,200 | 9 | 4.3 | * | | |
| 2½ hours | 46,000 | 10 | 2.7 | 6,800 | 16 | 3.5 | * | | |

* Empty.

swallowed—when the bacterial content of the food may be disregarded. It would naturally be expected that the greatest multiplication of bacterial and maximum numbers would be attained in the "interdigestive" phase, when the stomach is relatively at rest and the secretion of acid is at a minimum. Accordingly, therefore, the fasting contents should show the highest bacterial count. But such is not the case. As a matter of fact, the last fractions, whether saliva be removed or not, contain a far greater number of bacteria. Unquestionably the secretion of acid during the actual process of digestion together with the natural motility of the stomach would be expected to reduce the numbers of viable bacteria present at the beginning of the analysis. Contrary to this inference, the numbers are actually increased, and this is additional evidence that the continual swallowing of saliva

(which contains millions of bacteria per c c) is in reality the factor which determines the bacterial content of the stomach. Again, the fact that the bacterial numbers when saliva was removed were so small as to be negligible is significant when it is noted that the secretion of acid is without much influence, i. e., only 2 bacteria appear when the total acidity is as low as 12 and as high as 70. All the facts mentioned indicate that the saliva is the most important single factor in influencing the bacterial content of the stomach under the conditions employed. Similar results were obtained when these tests were made on a healthy normal person and on other manic patients having very low gastric acidity. The most important consideration, however, is that these patients having a very low acidity would be precisely the type of subjects in whom bacteria might gain a foothold and make the stomach a focus of infection. Judging from the results when saliva is removed, this is far from being the case.

Therefore, Cotton's[1] contention that the stomach is a focus of infection finds no substantiation after a critical inspection of the fundamental facts. His conclusions are based on results obtained by the Rehfuss method, and therefore are open to the following objections: 1. Repeated analyses by the Rehfuss method on the same person yield different curves and little constancy in bacterial species. 2. No correlation can be established between low acidity and high bacterial numbers or species since it has been shown that acidity is not the limiting factor in determining the bacterial content of the stomach during a fractional analysis by the Rehfuss method. 3. Saliva is the most important factor in influencing the bacterial content of the stomach (without gastric lesions) although the bacterial content of the food ingested must be considered.

Should the objection be raised that the bacterial content of the stomach is an academic one, the burden of proof falls on him who asserts that it is possible to determine infection in the lining of the stomach wall. Removal of the stomach is the only positive method yet known of proving whether or not such infection exists. The advantage of obtaining the knowledge in such a manner could scarcely be considered worth while from the standpoint of treatment. Therefore, infection in the stomach (without lesions) has been diagnosed by Cotton from results obtained by the Rehfuss method. Two possibilities are open. The first is that the infection is secondary and has been brought by means of the blood stream and lymph channels; the second is that it results from the swallowing of infected material. In either event

if the infection is "hidden" in the living stomach wall, the gastric contents on withdrawal will not show the bacteria in question. On the other hand, if the bacteria from the focus are being actively discharged into the stomach contents, if the infection has come by way of the blood or lymph stream, the number of bacteria found in the gastric contents should not be decreased by cutting off the flow of saliva. However, such is not the case, and it has been shown that the removal of saliva reduces the bacterial content. Consequently, it must be inferred that the bacteria in the gastric contents are introduced by the swallowing of saliva. It naturally follows, then, that there should be a multiplication of microorganisms when the stomach is at rest. However, it has been previously shown [2] that no such multiplication exists, even in stomachs having a low acidity.

It may be of interest in this connection to cite several cases of functional psychoses in which the patients showed approximately the same bacterial content, quantitatively as well as qualitatively, in the fasting contents and during analysis a considerable time after the primary foci were removed. Furthermore, the curves of acidity did not show any material improvement. The limitations imposed on any of the foregoing generalizations by virtue of the comparatively small number of persons under observation are fully realized, and only an intensive study of a great many cases can actually decide the points at issue. For the present the chain of evidence seems reasonably definite and points clearly to the conviction that the stomach (without lesions) cannot be considered a focus of infection on the basis of results obtained by the Rehfuss method of gastric analysis.

## SUMMARY

The following results were obtained by the Rehfuss fractional method of gastric analysis carried out on normal and psychotic persons. Subject to limitations and to the material under observation it was found that:

The organisms most frequently found in the stomachs of normal and psychotic persons were members of the staphylococcus, streptococcus, lactobacillus and yeast groups.

During an analysis, approximately the same types and numbers of bacteria were found in the stomach irrespective of high or low acidity fluctuations. This indicates that the gastric acidity is not the most important factor limiting the bacterial content of the stomach during a fractional analysis.

Streptococci were found associated with high, as often as with low, gastric acidity; consequently there seems to be no reason to attach undue importance to their presence or therefore to consider the stomach a focus of infection.

A method was devised for studying the influence of saliva on the bacterial content of the stomach. A striking reduction in numbers of bacteria occurred when the swallowing of saliva was thus reduced, indicating that saliva was a factor of considerable importance. This was even observed in subjects having a low gastric acidity contrary to expectation, if the stomach was to be considered a focus of infection.

The removal of primary foci of infection has not caused any material change in the gastric acidity, types or numbers of bacteria found in the patients examined.